TABLE 7

Setup for PCR reactions per sample

Assay Reagents	PCR mix (SNP)
PCR ProAMp mastermix with ROX	12.5 μL
20X Taqman assay working solution SNP1	1.25 μL
Total volume (per well)	13.75 μL

[0121] Example: For 10 samples genotyped for SNP, 1 positive control and 1 negative control, the final volume of the PCR Taqman master mix (SNP) will be 14× 14=193 μL (12 reactions and 2 extra to account for pipetting error):

[0122] For each sample or control, dispense 15 μL from the PCR mastermix for SNP being tested.

[0123] For each sample add 10 μL of extracted gDNA, sample to the well containing appropriate master mix for SNP.

[0124] For each positive control reaction, add 10 μL of positive control to the well containing SNP mastermix.

[0125] At least 1 positive control should be included with each mastermix to provide details on reaction efficiency.

[0126] For each negative control reaction, add 11.25 μL of negative control to a well contain SNP mastermix.

TABLE 8

Dispense volumes per reaction well of PCR

Mastermix + Internal Control	PCR mastermix (SNP)
PCR reaction mastermix Table 2	13.75 μL
Sample/ Control material template	Upto 11.25 μL *
Water	—
Final Volume	25 μL

* In case 20 ng of gDNA results in less than 11.25 μL, volume, add nuclease free water to bring up the total reaction volume to 25 μL

[0127] The plasticware should now be sealed with adhesive film then centrifuged briefly to bring the reaction mix to the bottom of the well and eliminate air bubbles. A non-optical seal can be used for this step. After centrifugation, transfer the plasticware to validated thermal cycler for amplication. Reference to the instrument manual should be made for instructions on setting up an amplification run. Amplification should be carried out according to instrument-specific parameters.

[0128] Thermal Cycler Conditions

[0129] Refer to the Quantstudio 5 Instruction manual for information on how to operate the Real-Time PCR instrument and perform data analysis and program the instrument following conditions described herein. It is important to visually inspect the amplification plots for each sample to ensure that the results recorded are due to true amplification and cannot be attributed to background noise recorded above the defined thresholds. Select the appropriate flourophore with each channel and assign to the relevant target.

[0130] Configure the Real Time PCR Instrument with the following settings:

[0131] Experiment type: Qualitative

[0132] Reagents used: TaqMan

[0133] Reagents Ramp Speed: Standard

[0134] Reaction volume: 25 μL

[0135] Passive Reference dye: ROX

TABLE 9

Thermal cycling conditions for the HairPGx test

Step	Temperature (° C.)	Time	Number of cycles	Data collection
Activation	95	15 min	1	off
Denaturation	94	30 s	45	off
Annealing/extension	60	60 s		on

TABLE 10

Detector channel used to detect the presence of the HairPGx target SNPs

	Green	Yellow	Orange	Red
Reporter Dye Channel	FAM	JOE	ROX	Cy5
Quencher	None	None	None	None
SNP 1	Allele 1	Allele 2		

[0136] Interpretation of Results

[0137] Internal Control

[0138] Detection of an internal control is not required with a positive result. In instances where the internal control has failed but the sample has been reported as positive for one of the HairPDx SNPs the result should be considered valid. In cases where the sample is reported as negative for all targets and the internal control is negative, the assay should be repeated using the same sample but diluted 1:10. If the internal control is then positive previous result was due to a handling error/PCR inhibition and new retest results should be reported. In cases where the internal control is still reported as negative after retesting then sample should be re-tested starting from extraction step.

[0139] Analyze the Experimental Data

[0140] Follow the instructions for data analysis based on the instrument used.

TABLE 11

Instructions for data analysis

Software	Features
Real-time instrument software	Instrument software View real-time trace data to aide genotype calling Data analysis varies depending on the real-time PCR system. See the instrument user guide for more information
TaqMan Genotyper software	Desktop software Create studies Overlay data from multiple plates View real-time trace data to aide genotype calling

(TaqMan ® SNP Genotyping Assays User Guide (Publication Number MAN0009593 Revision B.0)

[0141] Instructions for Using Quantstudio Desktop and Analysis Software for Making Automatic Calls.

[0142] Allelic discrimination plot (see FIG. **2**) can be viewed under the Results tab. In case there is no data displayed in the Results tab, click Analyze.

[0143] 1. Under the Results tab using the dropdown option, select Allelic Discrimination Plot.